measuring the amount of bioluminescence.

31. (New) The method according to Claim 30, wherein the contacting is performed in the presence of at least 0.001% cationic surfactant.

B11 cont'd

32. (New) The method according to Claim 30, wherein the bioluminescence reagent further comprises luciferin.

33. The method according to Claim 30, wherein the extracting is performed in the presence of a surfactant.--

SUPPORT FOR THE AMENDMENT

The specification is amended to remove brackets in the subtitles. Claims 14-33 are added. The addition of new Claims 14-33 is supported at pages 1-25 of the specification and in the original claims. No new matter is believed to be introduced by the amendment.

REMARKS

Claims 1-13 are cancelled in favor of new Claims 14-33. Favorable reconsideration is respectfully requested.

At the outset, Applicants thank Examiner Slobodyansky for indicating that the amendment would further favorable prosecution of the present application and for helpful comments during the discussion held on February 6, 2002.

The rejection to Claims 1 and 10-13 under 35 U.S.C. § 102(b) over Simpson et al is obviated by the cancellation of these claims. Further, Simpson et al neither describes nor suggests the isolated polypeptide recited in newly-added Claims 14-33.

The present application relates to a recombinant and mutagenized isolated polypeptide having luciferase activity which is derived from an insect belonging to the Order Coleoptera (See Claim 14). The claimed isolated polypeptide has improved luciferase activity in the presence of a surfactant compared to a luciferase in which a mutation has not been introduced (See Claim 14).

The luciferase disclosed in Simpson et al was purchased from Sigma Chemical Company (Sigma Catalog No. L-900). The attached pages of the Sigma's catalog (page 641) demonstrates that the luciferase disclosed in Simpson et al is a wild-type luciferase from American Firefly (Photinus pyralis). Thus, it is clearly different from the luciferase of the present application because it is not mutagenized. Further, Simpson et al fail to describe or suggest a recombinant and mutagenized isolated polypeptide having improved luciferase activity in the presence of a surfactant compared to a luciferase in which a mutation has not been introduced. Accordingly, Simpson et al fail to describe or suggest the claimed invention, and withdrawal of this ground of rejection is respectfully requested.

The rejection to Claims 1 and 4-13 under 35 U.S.C. § 102(b) over Tatsumi et al is obviated by the cancellation of these claims. Further, Tatsumi et al neither describes nor suggests the isolated polypeptide recited in newly-added Claims 14-33.

The luciferase disclosed in Tatsumi et al is a wild-type luciferase. Thus, it is clearly different from the luciferase of the present application because it is not mutagenized. Further, Tatsumi et al fail to describe or suggest a recombinant and mutagenized isolated

polypeptide having improved luciferase activity in the presence of a surfactant compared to a luciferase in which a mutation has not been introduced. Accordingly, Tatsumi et al fail to describe or suggest the claimed invention, and withdrawal of this ground of rejection is respectfully requested.

The rejection to Claims 1-13 under 35 U.S.C. § 102(e) over Hirokawa et al is obviated by the cancellation of these claims. Further, Hirokawa et al neither describes nor suggests the isolated polypeptide recited in newly-added Claims 14-33.

The basis for the Examiner's rejection is the subject matter related to SEQ ID NO: 14 of Hirokawa et al (see page 9, line 18, to page 10, line 5 of Office Action). Hirokawa et al was filed in the U.S. on July 8, 1998. Although Hirokawa et al does claim priority to Provisional Application Number 60/051,917 filed on July 8, 1997 (see column 1, lines 6-8, of Hirokawa et al), SEQ ID NO: 14 and its related subject matter in Hirokawa et al is not disclosed in Provisional Application Number 60/051,917. Copies of Provisional Application Number 60/051,917, the date-stamped filing receipt, and the USPTO filing receipt are attached for the Examiner's convenience. Therefore, the subject matter related to SEQ ID NO: 14 of Hirokawa et al is not entitled to priority under 35 U.S.C. § 119(e) to Provisional Application Number 60/051,917. Since there is not a priority date for subject matter related to SEQ ID NO: 14 of Hirokawa et al, this subject matter maintains an effective filing date of July 8, 1998.

Applicants respectfully submit herewith an English-language translation of the priority document, JP 9/361022, filed on December 26, 1997, establishing a perfection of priority of the present application. As a result of the perfection of priority, the priority date of the present application is December 26, 1997. Therefore, the priority date of the present

application is before July 8, 1998, which is the effective filing date of subject matter related to SEQ ID NO: 14 of Hirokawa et al. Accordingly, withdrawal of this ground of rejection is respectfully requested.

The rejection to Claims 1-13 under 35 U.S.C. § 112, second paragraph, is obviated by the cancellation of these claims. Accordingly, withdrawal of this ground of rejection is respectfully requested.

The rejection of Claims 1-13 under 35 U.S.C. § 112, first paragraph, is obviated by the cancellation of these claims. Further, new Claims 14-33 obviate this rejection for the reasons stated below.

Claim 14 relates to an isolated polypeptide, wherein said isolated polypeptide is a recombinant and mutagenized polypeptide having luciferase activity; said isolated polypeptide is derived from an organism belonging to Order Coleoptera, and said isolated polypeptide has improved luciferase activity in the presence of a surfactant compared to a luciferase in which a mutation has not been introduced. Claim 15 relates to a luciferase from an insect belonging the Family Firefly having sequence homology to a luciferase of Genji and Heike Firefly which are disclosed in examples of the present application. Claim 16 relates to luciferase that is resistant to a cationic surfactant. In Claim 17 relates to a luciferase resistant to benzalkonium chloride. In Claim 18, the above-mentioned luciferase resistance is clearly defined as "luciferase activity of said isolated polypeptide is not less than 89.3% in the presence of a surfactant compared to the luciferase activity in the absence of a surfactant." This definition is supported by Table 1 of the present specification (attached for the Examiner's convenience).

As described above, the claimed luciferase is clearly specified by defining an origin and by reciting "recombinant and mutagenized." Further, the invention specifies the surfactant and defines "resistance to a surfactant."

Further, Applicants found that a mutation at position 490 is useful for imparting a luciferase resistance to a surfactant by screening experiments described in detail below. A random mutation was introduced into the thermostable luciferase from Genji Firefly having lower resistance to surfactant. The degree of the improvement in luciferase resistance was unpredictable. Therefore, a luciferase originally having lower resistance to a surfactant makes it easier to evaluate even the slightest improvement in resistance within the parameters of the above-mentioned experiments. Moreover, a luciferase originally having lower resistance produces a precise evaluation of resistance even when impurities in the crude enzymes may make it difficult to evaluate the improvement in resistance when screening activity. For the reasons stated above, Applicants choose to not use the luciferase from Heike Firefly which naturally has high resistance to a surfactant. Accordingly, Applicants used a luciferase of Genji Firefly.

Crude enzymes were obtained from about 2,000 clones and an improvement in resistance to surfactant was screened and evaluated by measuring the change of light emission with the passage of time over one minute. In the screening step, this long-time period measurement (i.e. at least 1 minute) enabled the evaluation even the slightest improvement and resistance to surfactant. This avoids any imprecise evaluation of improvement in resistance that may occur due to impurities in crude enzymes at short-time measurements (i.e. less than 1 minute).

Further, a full nucleotide sequence was determined for two mutants that showed improved resistance to a surfactant during the above-mentioned screening. The sequencing results revealed that these two mutants had the same amino acid substitution, i.e., glutamic acid by lysine at position 490.

In light of the above, the scope of the claimed invention is enabling such that the skilled artisan reading the present application may now obtain a mutant luciferase having improved resistance to a surfactant by introducing a mutation into a recombinant luciferase according to the descriptions of the present application. In summary, Claims 14-33 are not drawn to an enormous genus of a luciferase both naturally occurring and man-made or a mutated luciferase having resistance to a surfactant as the Examiner contends.

The Examiner further contends that a skilled artisan cannot reasonably conclude that the Applicant had possession of the claimed invention at the time the instant application was filed. In contrast, as described above, the mutation at position 490 was commonly effective among luciferases having high homology to each other. Therefore, the mutation at position 490 is considered to be commonly effective in the improvement of luciferase resistance to a surfactant in other luciferases from an insect belonging to the Family Firefly having high homology to Genji and Heike Firefly luciferases. Accordingly, Applicants have demonstrated that they have possession of the claimed invention at the time the present application was filed, and withdrawal of this ground of rejection is respectfully requested.

The rejection of Claims 1 and 4-13 under 35 U.S.C. § 112, first paragraph, is believed to be obviated by the cancellation of these claims. Further, new Claims 14-33 reasonably provide enablement for the claimed invention. Applicants have disclosed a particular random screening process to obtain the claimed luciferase. This process is explained in the above-

mentioned arguments. This screening process reveals that a mutation at position 490 contributes to the claimed luciferase's improved resistance to a surfactant. In summary, the present specification puts forth and enables a process by which luciferase activity may be mutated and subsequently screened for activity in the presence and/or absence of a surfactant. Accordingly, Applicants have fully enabled the skilled artisan reading the present application in methods of a particular random screening process to obtain the claimed invention, and withdrawal of this ground of rejection is respectfully requested.

The objection to the specification is obviated by amendment. Accordingly, withdrawal of this ground of objection is respectfully requested.

Applicants respectfully submit that the present application is now in condition for allowance. Favorable reconsideration is respectfully requested. Should anything further be required to place this application in condition for allowance, the Examiner is requested to contact Applicants' Attorney by telephone.

Respectfully submitted,

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Marked-Up Copy
Serial No: 09/581,241
Amendment Filed on:
HEREWITH

IN THE SPECIFICATION

Page 5, please delete the paragraph beginning at line 1 and ending at line 1, and replace with the following paragraph.

--[[]Luciferase having resistance to surfactant[]]--.

Page 7, please delete the paragraph beginning at line 13 and ending at line 13, and replace with the following paragraph.

--[[]Method for producing mutant luciferase by genetic engineering techniques[]]--.

Page 10, please delete the paragraph beginning at line 12 and ending at line 12, and replace with the following paragraph..

--[[]Detection of intracellular ATP of the present invention[]]--.

Page 13, please delete the paragraph beginning at line 4 and ending at line 4, and replace with the following paragraph.

--[(]Method of preparing wild type luciferase derived from various firefly species[)]--.

Page 13, please delete the paragraph beginning at line 18 and ending at line 18, and replace with the following paragraph.

--[(]Method of determining luciferase activity[)]--.

Page 14, please delete the paragraph beginning at line 5 and ending at line 5, and replace with the following paragraph.

--[(]Method of determining surfactant-resistance[)]--.

Page 15, please delete the paragraph beginning at line 8 and ending at line 8, and replace with the following paragraph.

--[()]Production of gene encoding mutant luciferase HLK[)]--.

Page 16, please delete the paragraph beginning at line 15 and ending at line 15, and replace with the following paragraph.

--[()]Preparation of gene encoding mutant luciferase HIK[)]--.

Page 18, please delete the paragraph beginning at line 2 and ending at line 2, and replace with the following paragraph.

--[()]Changes in emission with time[)]--.

Page 19, please delete the paragraph beginning at line 2 and ending at line 2, and replace with the following paragraph.

--[()]Comparison of emission rate[)]--.

Page 20, please delete the paragraph beginning at line 6 and ending at line 6, and replace with the following paragraph.

--[()]Comparison of IC50[)]--.

IN THE CLAIMS

--Claims 1-13 are cancelled.

Claims 14-33 are new.--